






First records of *Chrysocyon brachyurus* (Illiger, 1815) (Mammalia, Carnivora) in the Gualeguay Department, Entre Ríos Province, Argentina

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Abstract

Maned Wolf, *Chrysocyon brachyurus* (Illiger, 1815), is the largest canid in South America. We present two new records of *C. brachyurus* in the Department of Gualeguay, Province of Entre Ríos, a road-killed specimen (32°59'52"S, 059°30'59"W) which we identified from a partial COI-gene sequence, and another individual identified from a photograph (32°57'12"S, 059°38'38"W). We also review and comment on recent reports of specimens recorded further south of the known range of the species in a conservation context.

Keywords

Canidae, COI, distribution boundary records, individual roadkill, molecular identification, *C. brachyurus*, recent expansion

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Introduction

Maned Wolf, *Chrysocyon brachyurus* (Illiger, 1815), is the largest canid in South America (Nowak and Paradiso 1983; Dietz 1984; Soler 2009a). Classified as a hypocarnivore (Wang et al. 2004), its diet includes a similar proportion of fruits and small mammals and birds (Aragona and Setz 2001; Amboni 2007; Rodrigues et al. 2007; Soler et al. 2014; Rodden et al. 2004; Queirolo and Motta-Junior 2007). The adults can weigh up to 30 kg and have

a length from head to tail of up to 132 cm (Nowak and Paradiso 1983; Soler 2009a). The pelage in adults vary from orange to golden red, and there is always a long mane and a characteristic white chin. The cubs have a much darker pelage and do not have a distinct mane or chin (Soler 2009a). The skull is elongated in dorsal and ventral view (especially in its rostral part), with sharp premolars and molars with wide surfaces to crush food.

Its limbs are long and end in conspicuous nails. The foot pads of fingers 3 and 4 of both limbs are fused in the proximal half (Fernández Salvador 1996), a trait shared with *Speothos venaticus* (Lund, 1842), a closely related species (Dietz 1985).

Molecular tools such as mitochondrial DNA (mtDNA) sequencing, in particular specific regions of the cytochrome oxidase subunit 1 (COI) gene, are commonly used to verify taxonomic identifications based primarily in morphology (Hebert et al. 2003; Borisenko et al. 2008; Luo et al. 2011; Paz et al. 2011).

Chrysocyon brachyurus occurs from northwestern Brazil, eastern Peru, and northern Bolivia to Rio Grande do Sul in southern Brazil and through the Paraguayan Chaco to northeast and central Argentina (Dietz 1984; Rodden et al. 2004; Queirolo et al. 2011). In Argentina, its presence has been recorded in the provinces of Corrientes, Formosa, Chaco, Santa Fe, Santiago del Estero, and northern areas of Córdoba and Entre Ríos (Cirignoli et al. 2019); it was also documented in Misiones (Massoia et al. 2012). In Entre Ríos Province, the species has been documented mostly in the northern part (Maranta 1998; Chebez 2005; Queirolo et al. 2011; Cirignoli et al. 2019). In recent years in Entre Ríos province, *C. brachyurus* has been recorded, mainly in news reports, in areas outside its known range, which are summarized in Table 1.

Chrysocyon brachyurus lives preferably in open or semi-open environments and particularly in grasslands (Rumiz and Sainz 2002; Mendez Coelho et al. 2008; Soler et al. 2011; Emmons 2012; Orozco et al. 2015). However, the species can be found frequently in forests and in fields under intense agro-livestock use (Dietz 1984; Michelson 2005; Soler et al. 2014; Orozco et al. 2015).

Today, following International Union for the Conservation of Nature (IUCN) criteria, *C. brachyurus* is classified as Near Threatened (Paula and DeMatteo 2015), while in Argentina it is Vulnerable (Cirignoli et al. 2019). However, a distinct, isolated subpopulation in northern Entre Ríos has been classified as Endangered. Although the species occurs in several protected areas (28 within Argentina), in Entre Ríos it has only been recorded from the Ayuí Grande Private Natural Reserve (Cirignoli et al. 2019).

Herein, we present two new records of *Chrysocyon brachyurus* from Entre Ríos, with taxonomic identifications based on morphology and molecular data. We also review and comment on the recent reports of specimens recorded further south of the known distribution of the species in a conservation context.

Methods

The study area is the Gualeguay Department in southern Entre Ríos Province (Fig. 1). This department is characterized by a flat, central plain, low hills in the north, and the Gualeguay and Paraná river delta in the south (Malvárez 1999). The entire department is characterized by abundant bodies of freshwater. The main river is the Gualeguay, which flows into the Paraná Guazú River. The Paraná Guazú River forms a delta at the southern end of the Gualeguay river basin (Pereyra et al. 2004; Quignard et al. 2013, 2017). This river and its many tributary streams are surrounded by wide, seasonally flooded plains, where there are several lagoons and oxbow lakes (Ormazábal et al. 2005; Margasin et al. 2020). Given this fluvial complexity, the area shows a diverse range of environments, which is reflected in the flora, fauna, and the diversity of the landscape (Bó and Malvárez 1999; Madanes et al. 2010; Apodaca et al. 2019). The main vegetal formation in our study area is the temperate grassland, with the grass genera *Axonopus*, *Paspalum*, *Digitaria*, *Schizachtrium*, and *Bothriochloa* (Poaceae) dominating (Bilenca and Miñarro 2004; Matteucci 2012). The grasslands alternate wetlands and forests of *Prosopis* spp. (Fabaceae), *Erythrina cristagalli* (Fabaceae), *Celtis ehrenbergiana* (Cannabaceae), and *Vachellia caven* (Fabaceae), among others (Apodaca et al. 2019), and many species of invasive exotic trees have been added in recent decades [e.g., *Gleditsia triacanthos* (Fabaceae), *Fraxinus* sp. (Oleaceae), *Melia azedarach* (Meliaceae), *Ligustrum lucidum* (Oleaceae)] (Quintana et al. 2005; Bortoluzzi et al. 2008; Torresín et al. 2013).

At the zoogeographic level, the area is located in the Entre Ríos sector of the Mesopotamian District (Ringuelet 1961), where species of the Pampas fauna [e.g., *Rhea americana* (Linnaeus, 1758), *Didelphis albiventris* Lund, 1840, *Lagostomus maximus* (Desmarest, 1817), *Cavia aperea* (Erxleben, 1777), *Dasypus hybridus* (Desmarest, 1804), *Conepatus chinga* (Molina, 1782), and *Leopardus geoffroyi* (d'Orbigny & Gervais, 1844)] are mixed with Mesopotamian representatives (*Salvator merianae* Duméril & Bibron, 1839, *Hydrochoerus hydrochaeris* (Linnaeus, 1766), *Dasypus novemcinctus* Linnaeus, 1758, *Cerdocyon thous* (Linnaeus, 1766), and *Procyon cancrivorus* (Cuvier, 1798), among others) (Quintana et al. 2005; Muzzachiodi 2007; Madanes et al. 2010). This diverse fauna is characteristic of the Pampa Ecoregion, Pampa Húmeda Subregion, and Mesopotamic Pampa Complex (Mateucci 2012). The main human activities in the study area are agriculture, particularly soybean and

Table 1. Summary of the records out of the distribution area of *Chrysocyon brachyurus* in news reports.

Date	Locality	Province	Commentary	Reference
Sep-2017	Pérez Millán, Ramallo	Buenos Aires	Captured and released later on San Alonso Island (Iberá Natural Reserve, Corrientes)	Ramallo Informa 2017
Jul-2017	El Porvenir, Florentino Ameghino	Buenos Aires	Video recording	Soler et al. 2017
Jun-2019	Trapiche, Coronel Pringles	San Luis	Video recording	Agencia de Noticias San Luis 2019
May-2020	Rosario, Rosario	Santa Fe		El Litoral 2020

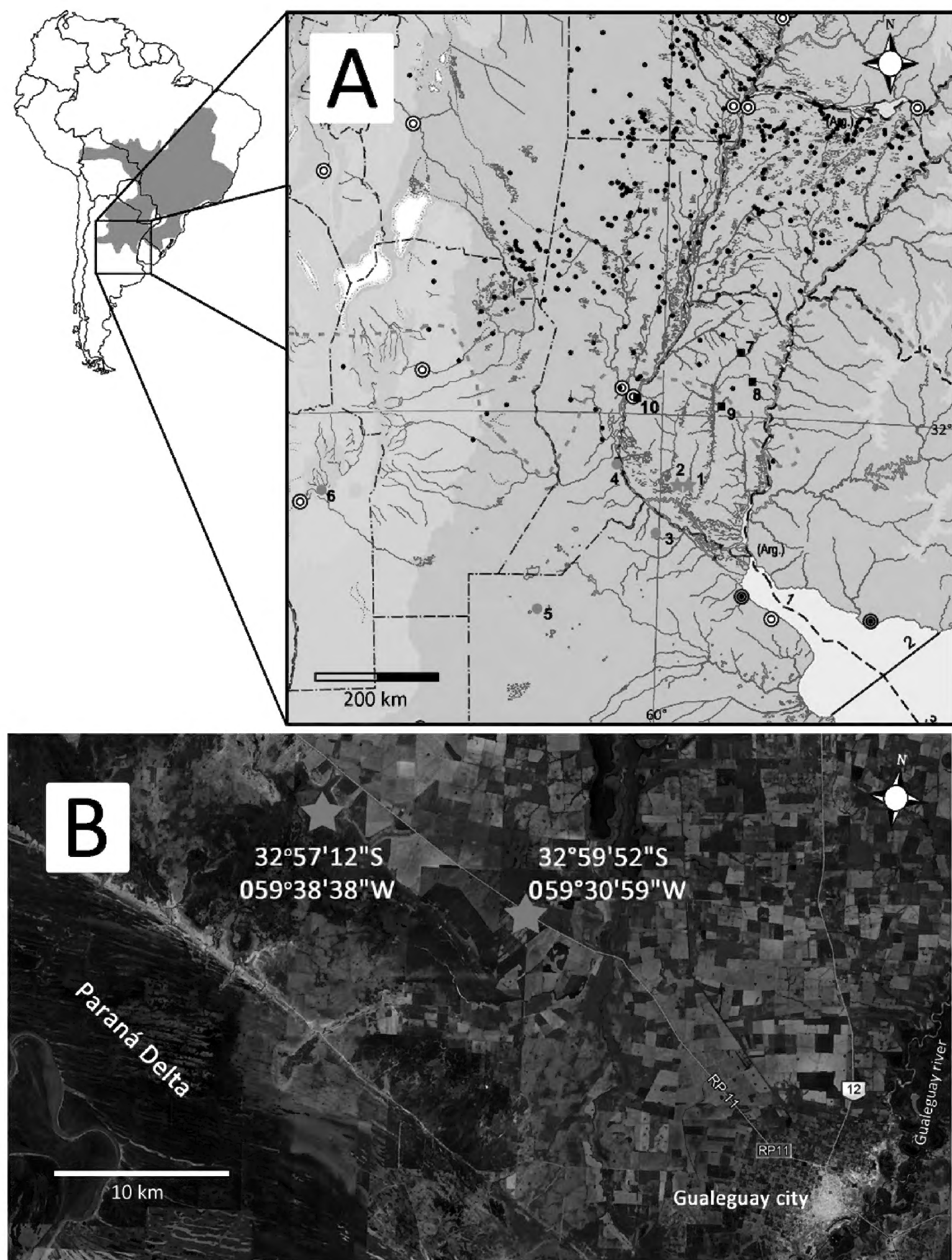


Figure 1. Location of the new records, previous records, and southern limit of the geographic distribution of *Chrysocyon brachyurus* in central Argentina. **A.** Map with recent records (last 30 years). Black dots = records from Pautasso (2009) and Queirolo et al. (2011). Red star and red dots = border and occasional records in the last 3 years: (1, 2) Gualeguay Department, Entre Ríos Province (new records); (3) Perez Millán, Buenos Aires Province; (4) Rosario, Santa Fe Province; (5) Florentino Ameghino, Buenos Aires Province (Soler et al. 2017); (6) Trapi-che, San Luis Province (Agenciasanluis 2019). Black squares = other recent records mentioned in the text within the Entre Ríos Province: (7) mouth of the Diego López stream, Federal Department; (8) San Salvador, Federal Department; (9) Villaguay, Federal Department; (10) Paraná city, Federal Department. Orange dotted lines = southern limits of the geographic distribution of *C. brachyurus* after Paula and Dematteo (2015). **B.** Satellite map of the area of the new records (yellow stars) in southern Entre Ríos Province.

corn crops, and cattle ranching. The lands are privately owned and currently there is no protected area in the Department of Gualeguay (Chebez 2005).

The first identification of the specimens was based on morphological traits, such as the pelage, limbs, and foot pads, and also the diagnostic large size of this canid. Samples of skin and hair (Sample no. 1303) were taken and deposited in the sample bank of the Centro

de Bioinvestigaciones (CeBio, UNNOBA-CICBA, Pergamino, Buenos Aires, Argentina) and preserved in 96% alcohol and kept at -20°C . For the molecular identification of the individual, the genomic DNA was extracted following the phenol-chloroform protocol (Sambrook and Russell 2006) and a fragment of the COI gene (approximately 710 bp) was amplified by polymerase chain reaction (PCR) using the primers: LCO-1490 (5'-GGTCAAC

AAATCATAAAGATATTGG-3') and HCO-2198 (5'-TA AACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). The amplification product was purified using 10U of Exonuclease I and 1U of FastAp thermosensible alkaline phosphatase and sequenced by Sanger sequencing in Macrogen Co. Ltd. (South Korea). The sequence obtained was viewed and edited manually using BioEdit v. 7.0.5 (Hall 1999). BLAST (Altschul et al. 1990) was performed to verify sequence similarity among our obtained COI sequence and other carnivore sequences deposited in the GenBank database (Table 2). Multiple alignment was made using the ClustalW algorithm in MEGA v. 6 (Tamura et al. 2013) and the phylogenetic tree was generated by the Bayesian inference method implemented in BEAST (Drummond et al. 2012). For the tree reconstruction, the following settings were used: HKY + G substitution model obtained from JModelTest v. 2.1.4 software (Darriba et al. 2012), a relaxed lognormal clock as molecular clock rate variation model and a 10,000,000 generations Monte Carlo Markov Chain length, sampling every 1,000. The trees were summarized with the maximum clade credibility (MCC) option from Tree Annotator v. 1.7.5 (Drummond et al. 2012) and the final tree was visualized in FigTree v. 1.4.0 (Rambaut 2012).

To compare our records with the current distribution (last 30 years) of *C. brachyurus*, we followed Pautasso (2009) and Queirolo et al. (2011), as well as the IUCN distribution map (Paula and Dematteo 2015).

Results

New records. ARGENTINA – **Entre Ríos Province** • Gualaguay Department, km 186 of Provincial Route No. 11; 32°59'52"S, 059°30'59"W; 7.VII.2019; Juan Sebastián Schweizer, Hernán Schweizer, and Nicolás Chimento legs.; road-killed individual; 1 ♀ adult; GenBank accession (COI): MW735445 • Gualaguay Department, 32°57'12"S, 059°38'38"W; 1.XI.2020; Hernán Schweizer obs.; 1 adult, sex indet.

Identification. The specimens had the typical orange pelage, with a long mane, and long, black limbs. In addition, the road-killed individual had limbs ending in strong, non-retractable nails and characteristic foot pads, with fingers 3 and 4 fused at their base on both front and back

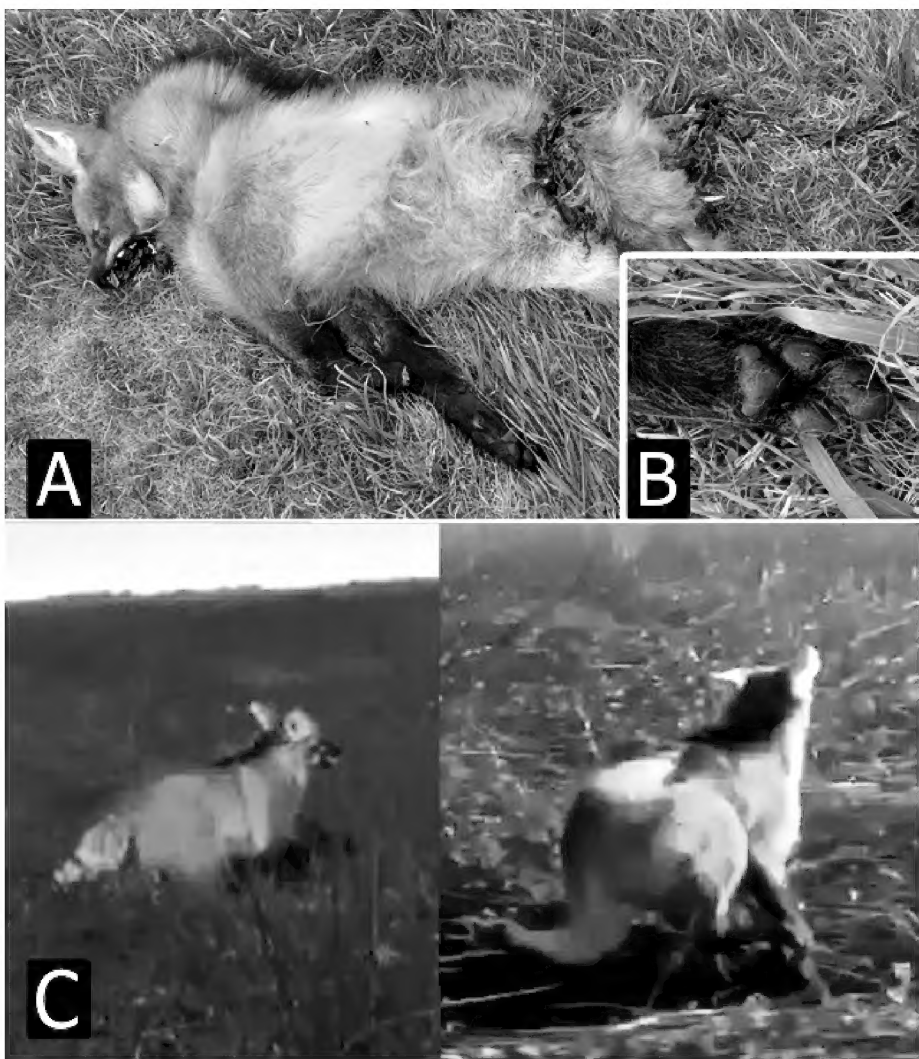


Figure 2. *Chrysocyon brachyurus* in Gualaguay Department, Entre Ríos Province, Argentina. **A, B.** Specimen recorded at 32°59'52"S, 059°30'59"W; road-killed at km 186 of Provincial Route No. 11. **A.** Left lateral view of the complete specimen. **B.** Plantar view of the left hindlimb. **C.** Two images captured from the filming of the specimen recorded at 32°57'12"S, 059°38'38"W.

limbs (Fig. 2), confirming their assignment to *Chrysocyon brachyurus*. The road-killed individual was 115 cm long from nose to the tip of the tail, 85 cm at the withers. The mane was distinct mane and there was a white chin typical of adult specimens.

At the genetic level, our molecular phylogenetic analysis grouped our specimen with a known sequence of *C. brachyurus*, separate from all other Neotropical canids, with a maximum posterior probability value equal to 1 (Fig. 3).

Discussion

In this work, we present the first formal records of *Chrysocyon brachyurus* in the Department of Gualaguay, southern Entre Ríos Province. We confirm the presence of this species near the southern limit of its current

Table 2. Sequences of COI mitochondrial gene from several Canidae species obtained from GenBank and their accession number.

Species	Origin	GenBank Accession numbers
<i>Chrysocyon brachyurus</i>	Argentina, Corrientes, Mburucuyá	MK321444
<i>Canis lupus</i>	—	AF028189
<i>Cerdocyon thous</i>	Argentina, Corrientes, Mercedes	MK321358
<i>Lycalopex culpaeus</i>	Argentina, Chubut, Cholila	MK321360
<i>Lycalopex fulvipes</i>	Chile, Araucanía, Gorbea	MK321398
<i>Lycalopex griseus</i>	Argentina, Río Negro, 40 km W Viedma	MK321353
<i>Lycalopex gymnocercus</i>	—	AF028201
<i>Lycalopex sechurae</i>	—	AF028202
<i>Lycalopex vetulus</i>	—	AF028196
<i>Speothos venaticus</i>	Brazil, Lagoa da Pedra, Januária, Minas Gerais	MK321384

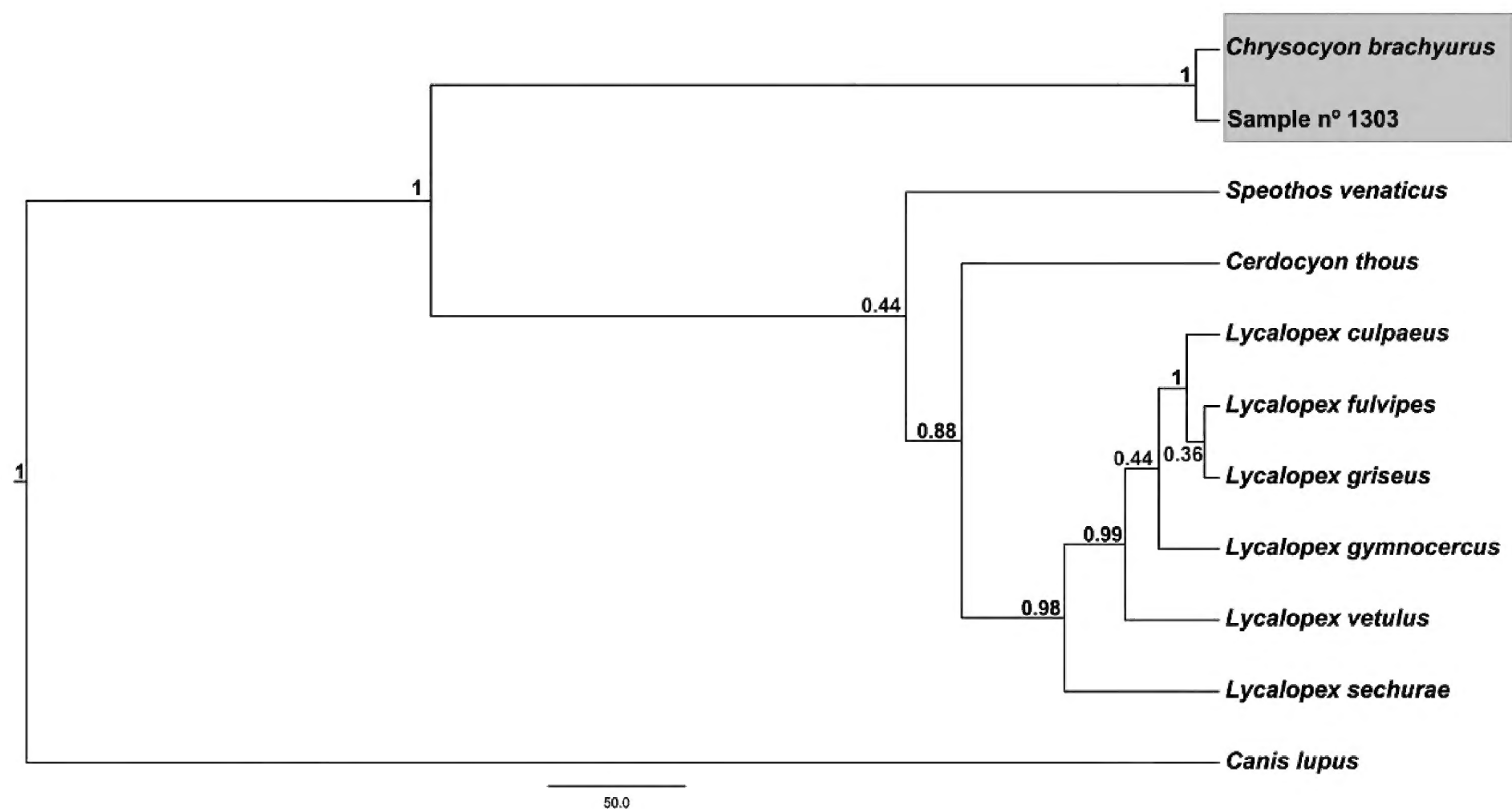


Figure 3. Bayesian phylogenetic tree obtained for the 710 bp fragment in the COI marker. Orange box shows the grouping of the road-killed individual (Sample no. 1303) with known sequences of *Chrysocyon brachyurus*. Posterior probability is expressed in the nodes.

distribution. Our records extend the area of occurrence of this species more than 170 km south from its previously known southernmost distribution limit (Paula and DeMatteo 2015).

During the last two decades, new occurrence data of *C. brachyurus* have shown that this species is expanding to previously unoccupied territories (Miatello and Cobos 2008; Pautasso 2009; Queirolo et al. 2011; Soler et al. 2014). Other recent data have verified the species at localities where only historical or archaeological records existed (García-Esponda et al. 2001; Prevosti et al. 2004). Until the late 1980s, the distribution of the species included the provinces of Corrientes, northern Santa Fe, eastern Chaco and Formosa, and southeastern Santiago del Estero (Beccaceci 1988; 1993). However, in the last 30 years, specimens have been recorded in western Santiago del Estero (Jayat et al. 1999; Richard et al. 1999), northeastern Córdoba (Haro et al. 2001; Miatello and Cobos 2008), and south-central Santa Fe (Beccaceci 1992; Pautasso and Fandiño 2008; Arnaudo and Pautasso 2009).

In the province of Entre Ríos, there are several reports of *C. brachyurus* from the second half of the 19th century, but specific localities are not mentioned, except for very broad areas (Ambrosetti 1887; Ripoll 1888; Cerutti 1990; Prevosti et al. 2004). More recently, records have been recorded from northern Entre Ríos (Muzzachiodi 2007; Queirolo et al. 2011), although no population-level studies have been conducted in this province. Perhaps for this reason, Entre Ríos is not included in the distribution of *C. brachyurus* by some authors (Díaz and Lucherini 2006; Queirolo et al. 2011; Orozco et al. 2015) or its presence in this province was questioned (Canevari and Vaccaro 2007; Parera 2018). There are several

records of specimens from the Department of San Salvador, in central Entre Ríos Province (Maranta 1998; Friedlander 2019), and Chebez (2008) mentioned other records from the departments of La Paz, Federal, and San Salvador, also in Entre Ríos, and referred to comments from residents' occasional records of Maned Wolf in the departments of Islas del Ibicuy and Villaguay on the Uruguay River. In Villaguay Department, the most recent record was of an adult female killed on a ranch by farmers (Azambuyo 2008; Fig. 1: square 8), which is the southernmost recent record in the province previous to our study. In October 2019, a Maned Wolf appeared prowling at night in the city of Paraná (ELONCE 2019). That specimen was captured and released a month later in a reserve in northern Entre Ríos Province (Unoentres 2019). Orozco et al. (2015) reported small populations in La Paz, Federal, and Feliciano Departments, as well as sightings in fields near Gualaguay River in the marsh environments of the Bracamonte Lagoon at the mouth of Diego López Creek. In the Federal Department, several records were cited in addition to others previously known (Prevosti et al. 2004; Chebez 2005; Orozco et al. 2015). As there are no specific records from southern Entre Ríos Province, we note the importance of Gualaguay Department record; it is the first thoroughly documented record of the species from the extreme south of Entre Ríos Province, separated by more than 170 km from the southernmost previously known records in the province at Villaguay and Paraná.

Our new record is added to sightings of *C. brachyurus* in the news reports at Rosario, Pérez Millán, and Trapiche (Table 1) and from personal communications, which represent individuals occurring beyond the previously known geographic range of the species. The

specimen sighted in a field in Paraje El Porvenir, Florentino Ameghino County, Buenos Aires Province (Soler et al. 2017; Table 1), is the southernmost record for the species in recent decades (Cirignoli et al. 2019). These scattered, southern records of *C. brachyurus* in Argentina possibly represent specimens that were previously in captivity and either intentionally or accidentally released into the wild. It is also plausible that our new records represent individuals of *C. brachyurus* which may have dispersed in the western Gualeguay river basin and looking for new territories.

Although *C. brachyurus* has some tolerance to anthropic environments (Dietz 1984; Michelson 2005; Soler et al. 2014; Orozco et al. 2015), the increasing fragmentation and degradation of the landscape as a result of ranching and monoculture is expected to increase the numbers of individuals dispersing in search of new territories (Arnaudo and Pautasso 2009).

The apparent recent expansion of the geographic range this species may be due to climate change, particularly more abundant rainfall and warmer temperatures (Prevosti et al. 2004), and some authors have mentioned that the increasingly southern distribution of several species of mammals (e.g., *Holochilus chacarius* (Thomas, 1906), *Dasypus novemcinctus*, *Hydrochoerus hydrochaeris*, *Cerdocyon thous*, and *Procyon cancrivorus*) (Voglino et al. 2004; Fracassi et al. 2010; Doumecq Miliau et al. 2012), birds, insects, and plants could correspond to the effects of climate change (Guerrero and Agnolin 2016). However, this may not be the most plausible explanation for *C. brachyurus*, as it is historically inhabited the Pampean Region in past centuries, where the climate was colder and more arid than it is today (Politis 1984; Deschamps et al. 2003; Tonni 2006, 2017). *Chrysocyon brachyurus* suffered a strong retraction in its geographic range (Queirolo et al. 2011; Soler et al. 2015b) at the end of the 19th century and the beginning of the 20th century (Prevosti et al. 2004), but now, the expansion of its range could be in search of animal prey (e.g., *Holochilus* spp., *Dasypus* spp., and *Cerdocyon thous*) and plant food (e.g., *Pouteria* spp., *Solanum* spp.) (Queirolo and Motta-Junior 2007; Rodrigues et al. 2007), instead of dispersal in search of warmer and more humid places. Many of the species that form the diet of *C. brachyurus* are being recorded increasingly farther to the south in the last decades (e.g., Voglino et al. 2004; Fracassi et al. 2010; Guerrero and Agnolin 2016). However, other hypotheses are possible and should not be discounted, such as less hunting due to better laws and conservation actions, or greater conservation awareness, or even the impacts of agriculture.

Finally, the poor availability of information on the distribution of *C. brachyurus*, especially along its southern limits, does not allow us to assert that the new records represent recent expansions of the species. Instead, these reports may be vagrant specimens or small, isolated populations, or may be due to greater attention recently given to this species by researchers (Pautasso and Fandiño

2008). More study, specifically long-term monitoring, is needed, and measures to protect against potential human conflict need to be in place.

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Authors' Contributions

Conceptualization: GPF. Data curation: MEM, NRC. Formal analysis: MEM, NRC, GPF. Funding acquisition: GPF. Methodology: MEM, NRC. Project administration: GPF. Validation: MEM, NRC, GPF. Writing – original draft: MEM, NRC, GPF. Writing – review and editing: MEM, NRC, GPF.

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